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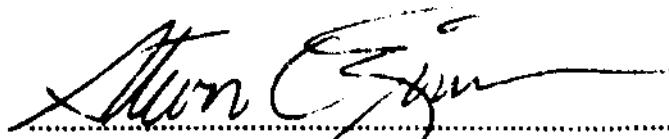
NATHANIEL S. FINNEY

ENTITLED DESIGN AND SYNTHESIS OF A

BIOMIMETIC CHYMOTRYPSIN MODEL

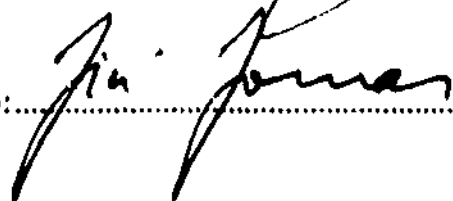
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DEGREE OF BACCALAUREATE OF SCIENCE



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Instructor in Charge

APPROVED:



HEAD OF DEPARTMENT OF CHEMISTRY

**Design and Synthesis
of a Biomimetic Model of Chymotrypsin**

Thesis

**submitted as partial fulfillment of the requirements
for the degree of Baccalaureate of Science in Chemistry.**

**College of Liberal Arts and Sciences
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Research Advisor: Steven C. Zimmerman

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Introduction and Background

Since the determination of its tertiary structure in 1968¹, the mammalian serine protease chymotrypsin has been the focus of extensive study, through both direct methods and the development of model systems. In addition to its native function of polypeptide amide hydrolysis, chymotrypsin has been shown to hydrolyze esters *in vitro*. X-ray crystallographic data^{2,3,4} have revealed three amino acids in the catalytic site that are critical to the catalytic function of the enzyme: Histidine-57, Aspartate-102, and Serine-157. Such studies have further demonstrated that these amino acids are hydrogen bonded in the ground state of the enzyme, as shown in Figure 1.⁵

The most widely accepted mechanism for the enzyme's hydrolytic activity was first proposed by Hunkapiller in 1973.⁶ In this 'charge relay system,' Asp-102 is the ultimate proton acceptor, deprotonating the imidazole of His-57, which functions as a general base, activating and deprotonating the hydroxyl group of Ser-195, which simultaneously attacks the carbonyl of the ester or amide substrate (See Fig. 2). Chemical modification of the readily accessible histidine and serine residues in question confirmed their importance to the hydrolytic activity of the enzyme. Chemical blocking of either amino acid completely deactivated the enzyme.^{7,8} Unfortunately, the 'buried' aspartate could not be readily modified, and as such, its role in the catalysis was unclear for some time.

Direct evidence against the involvement of Asp-102 in the enzymic catalysis has been provided primarily by NMR data. ^{15}N NMR studies indicate that both Asp-102 and His-57 have normal pK_a values.⁹

This finding is incompatible with their involvement in general base catalysis, in which they should exhibit higher pK_a values than usual.

^1H NMR investigation has shown that in the ground state of the enzyme, there is effectively no proton transfer from His-57 to Asp-102.¹⁰ It should be noted that this spectroscopic data pertains to the enzyme in the absence of substrate. It is conceivable that, on binding a substrate, the enzyme undergoes significant conformational change, and the active site no longer resembles the ground state structure indicated by the NMR analysis.

The strongest biochemical evidence in favor of the involvement of Asp-102 comes from the recent work of Craik.¹¹ Using the technique of site directed mutagenesis on trypsin, an enzyme closely related to chymotrypsin, they replaced the aspartate residue of the active site with an asparagine residue, eliminating the negative charge without effectively altering the size of the amino acid side chain. X-ray crystallographic structures¹² of the mutant enzyme showed the direction of hydrogen bonding of the critical amino acid triad to be completely reversed, Asn-102 acting as a proton donor rather than acceptor, as illustrated in Figure 3. Extensive kinetic studies demonstrated the mutant to be some 25,000 times less active at physiological pH than the native enzyme.¹¹ The major flaw in this convincing evidence is that the X-ray structures did indicate some minor perturbation of the binding

site as a result of the asparagine substituent, and it is not known to what extent this effects the activity of the mutant enzyme.

In addition to the study of serine proteases by direct methods, a number of organic models of the catalytic triad have been constructed to provide indirect information about the mechanism of catalysis. Most notable are the models of Bruice¹² and Bender¹³ (Figures 4 and 5, respectively), both of which are analogs of the Asp-102/His-57 general base couple that use water in place of Ser-195. Neither model demonstrates substantial catalytic activity for the carboxylate in the hydrolysis of esters, a surprising result, considering they seem ideally arranged to make use of the 'charge relay system.'

In an earlier paper on entropic effects in enzymic catalysis,¹⁵ Bruice ascribed the incredible rate enhancement of enzymes to three general effects. The first, proximity, takes into account the advantage of intramolecular reaction over intermolecular reaction. The second, desolvation, takes into account the fact that, in the active site of an enzyme, the reactants are already essentially desolvated, and there is no need to form an encounter complex prior to reaction (See Fig. 6). The third, orientation, is the observation that enzymes are structurally designed to hold all of the reactants in precise, nearly ideal, positions for maximum reactivity. While both of the described models show the desired property of reactant proximity, we believe they lack the other two properties necessary for catalytic rates approaching those of enzymes. On inspection, it seems that neither model guarantees desolvation of the reactants, due in both

cases to flexibility of the carboxyl-bearing side chain. Additionally, Gandour has proposed that both models are, in fact, orientationally flawed, as both use the anti lone pair of the carboxylate while enzymes use the syn lone pair.¹⁶ He has estimated that the syn lone pair is 10^3 - 10^4 times more basic than the anti lone pair, based on determination of the syn/anti equilibrium in protonated carboxylic acids.

In a broad review of general acid/general base catalysis, Kirby has noted that no general base catalysts exist exhibiting an effective molarity of greater than 80 M.¹⁷ This is particularly confounding when the enormous catalytic rates of enzymes are taken into account. In the one observed case of general acid catalysis exhibiting an EM of greater than 80 M, the large rate enhancement has been ascribed to the formation of a strong hydrogen bond in the product and a correspondingly low residual entropy¹⁷. The biomimetic models discussed above appear, on inspection, to lack this necessary driving force.

Taking into consideration the background information detailed above, a new charge relay model compound, having the phenanthro[9,10]imidazole structure of 3, was designed. After construction of CPK models, it seemed that models of this sort would possess the desired reactant proximity to a greater degree than previous models, would rigidly enforce orientational constraints, and would guarantee the required desolvation. Additionally, compounds of this sort should exhibit strong hydrogen bond formation throughout the catalytic cycle (See Fig. 7).

While the synthesis of the initial target compound 3, and the accompanying general route, was being worked out by a graduate student in our group, it seemed that in addition to making a good reference compound for kinetic studies of 3, compound 1 would make a valuable, if somewhat simpler, model in its own right. Most importantly, the compound had the advantage of being symmetric, allowing the development of a relatively short synthetic route. Once the synthesis of 1 and two related compounds was complete, and the initial synthetic route to asymmetrically substituted phenanthro[9,10]imidazoles proved impractical, attempts were made to develop a new route to these compounds.

Results and Discussion

Overall synthetic simplicity was the initial appeal of the synthesis of 1, as outlined in Scheme 1. 2,2'-Dimethoxybenzoin, 5, (*o*- anisoin) was prepared from commercially available *o*-anisaldehyde by the classical benzoin condensation method of Irvine,¹⁸ giving a 32% yield after recrystallization. Following the procedure of Bredereck,¹⁹ the *o*-anisoin was readily converted to the 4,5-diaryl imidazole, 6, by condensation with formic acid and formamide in DMF in a 72% yield. An early survey of the literature had revealed that a wide variety of diaryl imidazoles could be converted to phenanthro[9,10]imidazoles by photocyclization^{20,21,22}, although the procedure had not been applied to 4,5-di(2-methoxyphenyl) imidazole. After some experimentation with the photolysis solvent, methanol was selected, and 6 was cyclized to 7 in a disappointing 25% yield. As it turned out, the major byproduct, also isolated in 25% yield, was the demethoxylated compound 8. This result was not entirely unanticipated, as Sargent has reported a similarly substituted phenanthrene to undergo a photocyclization giving 35% of the desired product and 11% of the demethoxylated material.²³ When it was determined that the ratio of the two photolysis products was not particularly sensitive to the concentration of substrate, the concentration of iodine, or the temperature of reaction, we gained an interest in isolating 8 as a useful reference compound. The photolytic reaction, although aesthetically appealing, proved to be very capricious, and yields

were often poor. Phenanthrene imidazole 8 was neatly deprotected by following the procedure of McCarthy, heating the compound in DMSO with excess sodium cyanide to give the 2-hydroxy phenanthro[9,10]imidazole 9 in 65% yield.²⁴ The deprotection of compound 7 was somewhat less successful, giving roughly a two to one ratio of 1, the full diol, and 2, the mono methyl ether, and a large portion of starting material. Fortunately, 2, much like 9, will make an interesting reference compound.

During development and completion of the synthesis for symmetric phenanthrene imidazoles outlined above, a number of factors decreed that the previous route being studied for the synthesis of related asymmetric compounds (See Scheme 2.)²⁵ be abandoned. As in the symmetric route that it inspired, the photocyclization of 17 to form 18 was a difficult reaction, giving poor and inconsistent yields. Osmium tetroxide turned out to be impractically expensive in addition to being very difficult to work with, although it oxidized phenanthrene 18 to diol 19 in good yield. Most disappointingly, the methyl group of phenanthrene 21 proved almost impossible to functionalize, making the target compound, 3, completely inaccessible.

As a result of the aforementioned difficulties, we became interested in developing a new route to asymmetrical phenanthroimidazoles. It seemed that the general route illustrated in Scheme 3 would provide the desired products, without being longer than that which was abandoned, and would avoid the troublesome photocyclizations and oxidations of the earlier route.

Using the simplest asymmetric case possible, a pilot synthesis was initiated. Commercially available *o*-nitrotoluene was converted in exceptional yield to the *o*-nitrobenzylbromide, 10, using a standard light catalyzed bromination method. Phosphonium salt 11 was produced in excellent yield by stirring 10 with triphenylphosphine in chloroform.²⁶ A standard Wittig reaction with *o*-anisaldehyde provided unsymmetrical stilbene 12 in good yield,²⁶ with *cis* and *trans* isomers being readily separable by chromatography. The seemingly straightforward epoxidation²⁷ of 12 to 13 proved rather troublesome. It would seem, based on ¹H NMR coupling constants, that the *trans* stilbene cannot be converted into the epoxide with great facility, although the *cis* isomer reacts readily. Additionally, the product epoxide was difficult to purify, although it proved to be relatively stable to chromatography on silica. (See experimental for details.) When it was determined that the epoxide could not be oxidatively opened to the corresponding α -hydroxy ketone by the method of Swern and Khuddus,²⁸ it was decided to open it to the diol²⁹ and proceed along known lines from there. Research has currently halted at this step, the hydrolysis of 13 to diol 14. Although this was thought of initially as being the easiest step in the synthesis, to date it has proven impossible to cleanly open the epoxide, and the majority of attempted reactions have produced a multitude of products. Should this barrier be surmounted, the diol will be oxidized with acetic anhydride in DMSO to the dione, 15, a reaction with good precedent.³⁰ The dione will be converted to the imidazole, 16, by condensing it with hexylamine and ammonium

acetate in refluxing acetic acid.²² Reduction of the nitro group³¹ followed by a straightforward Pschorr oxidative ring closure³² should provide the target compound, 1.

Experimental

1,2-Di-(2-methoxyphenyl)-2-hydroxyethanone (5) To liquified o-anisaldehyde (30 g, 220.4 mmol) was added 1:1 water:ethanol (90 ml). The mixture was swirled to dissolve as much anisaldehyde as possible (Dissolution was never complete). Potassium cyanide (3.01 g, 46.28 mmol) was added, and the reaction mixture was immersed in an oil bath at 115° C. The initially cloudy yellow mixture changed to a darker, clear yellow after 2-3 minutes of heating. After refluxing 3 1/2 hours, the oil bath was removed, and the flask plunged into a ice/saltwater slurry. The solution was stirred with a glass rod until a gummy yellow solid began to form. Solid filtered off after standing in ice bath 30 minutes, and rinsed with cold water (10 ml). The yellow material was recrystallized from diethyl ether (450 ml), with hot filtration, to produce 9.60 g (32%) of 5 as a white crystalline solid: mp 100-101.5° (Literature mp 100-101° C). ¹H NMR (300 MHz, CDCl₃) δ 3.71 (s, 3H), 3.72 (s, 3H), 4.47 (d, J = 5.45 Hz, 1H), 6.10 (d, J = 4.98 Hz, 1H), 6.81 (m, 3H), 6.92 (t, J = 7.39 Hz, 1H), 7.16 (m, 2H), 7.37 (t, J = 8.13 Hz, 1H), 7.69 (d, J = 7.69 Hz, 1H).

4,5-Bis-(2-methoxyphenyl)-imidazole (6) Benzoin 5 (29.87 g, 109.8 mmol) was dissolved in a mixture of dimethylformamide (380ml), formamide (560ml), and formic acid (190ml). The resulting solution was heated in a 150° C oil bath for 22 hours. The solvent was removed at reduced pressure, leaving a light yellow solid. The

solid was slurried with cold water (70 ml) and filtered, affording 22.10 g (72%) of light yellow material: mp 191.5-193° C (Literature mp 193° C). ¹H NMR: (300 MHz, CDCl₃) δ 3.73 (s, 6H), 6.89 (t, J = 3.78, 2H), 6.94 (d, J = 8.43, 2H), 7.27 (m, 4H), 7.77 (s, 1H).

2-Methoxyphenanthro[9,10]imidazole (8) and 2,8-dimethoxyphenanthro[9,10]imidazole (7) A solution of diaryl imidazole 6 (2.5 g, 8.9 mmol) and iodine (0.38 g, 1.5 mmol) in methanol (2000 ml) was irradiated with an unfiltered 450W medium pressure mercury arc lamp for 4 hours. Most of the solvent was removed, and the remainder (150 ml) was diluted with methylene chloride (800ml). The solution was extracted with 10% sodium thiosulfate (500 ml), and the aqueous layer rinsed with methylene chloride (150ml). The organic layer was dried with magnesium sulfate and evaporated. The resulting brown solid was chromatographed (40 mm OD column, 8% methanol / 92% methylene chloride), yielding 1.15 g (46.3%) of 7 and 0.59 g (26.6%) of 8 as faintly pink powdery solids. 7: mp 188-191° C, dec. IR: 1579.9, 1487.3, 1458.4, 1408.2, 1323.3, 1257.7, 1120.8, 1024.3 cm⁻¹. ¹H NMR: (300 MHz, CDCl₃) δ 4.14 (s, 6H), 7.13 (d, J = 7.92 Hz, 2H), 7.53 (t, J = 8.17, 2H), 8.25 (s, 1H), 8.32 (d, J = 8.41 Hz, 2H). ¹³C NMR: (500 MHz, d₆-DMSO) δ 55.7, 108.0, 115.7, 116.3, 116.5, 125.4, 129.0, 138.1, 155.0. MS (EI) m/z 278. 8: mp 207-210° C, dec. IR: 1610.8, 1576.0, 1483.4, 1452.6, 1414.0, 1354.2, 1329.1, 1253.9, 1120.8, 1091.8, 1010.8 cm⁻¹. ¹H NMR: (300MHz, d₄-MeOH) δ 4.16 (s, 3H), 7.05 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 8.17 Hz, 1H), 7.50 (t, J = 7.91 Hz,

1H), 7.59 (t, $J = 7.74$ Hz, 1H), 8.09 (s, 1H), 8.20 (d, $J = 8.45$ Hz, 1H), 8.46 (d, $J = 7.98$ Hz, 1H), 8.57 (t, $J = 8.35$ Hz, 1H). ^{13}C NMR: (500 MHz, d_6 -DMSO) δ 55.6, 107.1, 113.5, 115.9, 121.7, 123.5, 124.1, 124.9, 125.5, 127.2, 127.5, 129.0, 136.1, 138.9, 154.3. MS (EI) m/z 248.

1,8-Dihydroxy-phenanthro[9,10]imidazole (1) and 1-hydroxy-8-methoxy-phenanthro[9,10]imidazole (2) Imidazole 7 (1.30 g, 7.29 mmol) and sodium cyanide (1.79 g,

36.45 mmol) were dissolved in DMSO (20 ml) and heated to 175°C under a nitrogen atmosphere. After stirring for 21 hours, the dark brown reaction mixture was allowed to cool, and the DMSO was removed by kugelrohr distillation. The resulting dark brown solid was partitioned between water (100 ml) and 10% methanol / 90% methylene chloride (100 ml). The organic layer was dried with magnesium sulfate and stripped to give a light tan powder.

Chromatography (60 mm OD column, 3% methanol / 97% methylene chloride) gave 0.502 g (27.3%) of 1 and 0.370 g (14.0%) of 2. 1: mp $>230^\circ\text{C}$. IR: 3406.7, 3279.4, 2924.4, 1578.0, 1448.7, 1273.2, 1257.7 cm^{-1} . ^1H NMR: (300 MHz, d_4 -MeOH) δ 7.07 (d, $J = 7.82$ Hz, 2H) 7.43 (t, $J = 8.08$ Hz, 2H), 8.13 (s, 1H), 8.19 (d, $J = 1.43$). ^{13}C NMR: (500 MHz, d_6 -DMSO) δ 110.5, 111.0, 115.0, 116.0, 125.5, 126.0, 137.0, 138.0, 155.0. MS (EI) m/z 250. 2: mp $205\text{--}208^\circ\text{C}$. IR: 3329.6, 1579.9, 1489.2, 1446.8, 1417.9, 1329.1, 1257.7, 1124.6, 1051.3 cm^{-1} . ^1H NMR: (300 MHz, d_4 -MeOH) δ 4.16 (s, 3H), 7.07 (d, $J = 8.05$ Hz, 1H), 7.22 (d, $J = 8.03$ Hz, 1H), 7.45 (t, $J = 8.23$ Hz, 1H), 7.54 (t, $J = 8.24$ Hz, 1H), 8.17 (s, 1H), 8.18 (d, $J = 3.1$ Hz, 1H), 8.33 (d, $J = 8.31$ Hz, 1H). ^{13}C

NMR: (500 MHz, d_6 -MeOH) δ 55.7, 107.6, 111.6, 113.3, 113.4, 114.0, 114.7, 116.5, 122.3, 126.2, 128.4, 129.2, 134.9, 137.6, 154.2. MS (EI) m/z 264.

1-Hydroxyphenanthro[9,10]imidazole (9) Imidazole **8** (1.00 g, 4.0 mmol) and sodium cyanide (1.0 g, 20.0 mmol) were dissolved in DMSO (20 ml). After stirring at 170° C for 21 hours, the dark brown solution was cooled, and the DMSO removed by kugelrohr distillation. The resulting brown solid was run through a short silica plug (40 mm OD, 6% methanol / 94% methylene chloride) to remove polar impurities. The brown oily material obtained was dissolved in a minimum of methylene chloride (25 ml) and diethyl ether (75 ml) was added with swirling. After standing in a freezer for 12 hours, 0.65 g (69.4%) light gray and white flecked crystals were collected: mp 227-229° C. IR: 1616.5, 1578.0, 1485.4, 1446.8, 1390.8, 1352.3, 1333.2, 1273.2, 1116.9 cm^{-1} . 1H NMR: (300 MHz, d_4 -MeOH) δ 7.09 (d, J = 7.82 Hz, 1H), 7.45 (t, J = 8.13 Hz, 1H), 7.57 (t, J = 7.43 Hz, 1H), 7.63 (t, J = 7.24 Hz, 1H), 8.14 (s, 1H), 8.23 (d, J = 8.39 Hz, 1H), 8.71 (d, J = 8.22 Hz, 1H). ^{13}C NMR: (300 MHz, d_6 -DMSO) δ 111.7, 114.6, 122.0, 124.6, 124.7, 125.3, 125.4, 125.5, 126.1, 127.5, 127.7, 127.8, 129.4, 138.5. MS (EI) m/z 234.

***o*-Nitrobenzylbromide (10)** To a solution of *o*-nitrotoluene (7.50 g, 54.70 mmol) in carbon tetrachloride (150 ml) was added *N*-bromosuccinimide (9.64 g, 54.20 mmol). A 150W unfiltered light bulb was placed beneath the flask containing the suspension, and the

bulb and flask wrapped in aluminum foil. The bulb was turned on, and the mixture allowed to reflux for 40 minutes. The reaction was cooled to ambient temperature, and the succinimide filtered off. The solvent was evaporated to afford a yellow oil, which was chromatographed (60 mm OD, 20% methylene chloride / 80% petroleum ether) to give 11.65 g (98.6%) of a yellow oil. The oil solidified on standing in a freezer overnight, producing a yellow solid: mp 46-47° C (Literature mp 45-48° C).

o-Nitrobenzyltriphenylphosphoniumbromide (11) A solution of *o*-nitrobenzylbromide 10 (3.00 g, 13.89 mmol) and triphenylphosphine (3.64 g, 13.89 mmol) in chloroform (30 ml) was stirred in a dry atmosphere for 36 hours. Diethyl ether (30 ml) was added, briefly forming a two phase system which became homogeneous on standing. After standing overnight, and being placed in a freezer for several hours, the yellow and white crystals were filtered off. The crystals were dissolved in hot ethanol (10 ml) and addition of diethyl ether (1 ml) initiated formation of 6.28 g (94.6%) of uniform light yellow crystals: mp 161° C (Literature mp 161-162° C).

Cis- and *trans*-2-nitro-2'-methoxystilbene (12) A solution of *o*-nitrobenzylphosphoniumbromide 11 (8.50 g, 17.77 mmol) and *o*-anisaldehyde (2.47 g, 18.13 mmol) in dry acetonitrile (90 ml) was heated to reflux in a dry atmosphere. Triethylamine (10.66 ml) was added to the yellow solution in four equal portions over 30 minutes,

bulb and flask wrapped in aluminum foil. The bulb was turned on, and the mixture allowed to reflux for 40 minutes. The reaction was cooled to ambient temperature, and the succinimide filtered off. The solvent was evaporated to afford a yellow oil, which was chromatographed (60 mm OD, 20% methylene chloride / 80% petroleum ether) to give 11.65 g (98.6%) of a yellow oil. The oil solidified on standing in a freezer overnight, producing a yellow solid: mp 46-47° C (Literature mp 45-48° C).

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each addition causing a color change to violet that faded rapidly. After 8 hours, the reaction was cooled and the solvent removed under reduced pressure. The resulting orange oil was dissolved in methylene chloride (50 ml) and evaporated onto silica gel (15 ml). Chromatography (50 mm OD, 25% methylene chloride / 75% petroleum ether) gave a mixture of stilbenes (3:1 cis:trans by NMR) weighing 4.54 g (84.7%), and whose ^1H NMR spectrum was identical to that of a known sample.

1-(2-Nitrophenyl)-2-(2-methoxyphenyl)ethyleneoxide (13) Stilbene 12 (7.82 g, 30.60 mmol) and mCPBA (7.70 g, 36.8 mmol) were dissolved in chloroform (120 ml) and the yellow solution refluxed, with a drying tube, for eight hours. Although TLC (50% methylene chloride / 50% petroleum ether, 0.20mm silica plate) indicated the presence of the trans-stilbene, the cis isomer was almost completely consumed, and prolonged heating did not promote further reaction. The reaction was cooled to room temperature and extracted with aqueous sodium hydroxide (1 M, 1x80 ml) and water (1x80 ml). The organic layer was dried and the solvent evaporated to give a red oil which could not be purified or chromatographed without substantial decomposition. The material was finally dissolved in methylene chloride (60 ml) and evaporated onto magnesium sulfate (10 g). Chromatography (60 mm OD, cold 30% methylene chloride / 70% petroleum ether) gave 2.24 g (27%) of a yellow solid: mp 105-107° C. IR: 1531.7, 1338.8, 1230.7 cm^{-1} . ^1H NMR: (300 MHz, CDCl_3) δ 3.72 (s, 3H), 4.63 (d, $J = 4.55$ Hz, 1H), 4.97

(d, $J = 4.54$ Hz, 1H), 6.64 (d, $J = 7.86$ Hz, 1H), 6.97 (d, $J = 7.36$ Hz, 1H), 7.04 (t, $J = 7.29$ Hz, 1H), 7.25 (t, $J = 7.49$ Hz, 1H), 7.46 (t, $J = 7.26$ Hz, 1H), 7.61 (d, $J = 7.54$ Hz, 1H), 7.88 (d, $J = 8.54$ Hz, 1H). ^{13}C NMR: (300 MHz, CDCl_3) δ 55.5, 57.0, 58.5, 109.5, 119.5, 122.5, 124.5, 127.5, 128.5, 129.0, 130.0, 133.0, 158.0. MS (EI) m/z 271.

Figures and Schemes

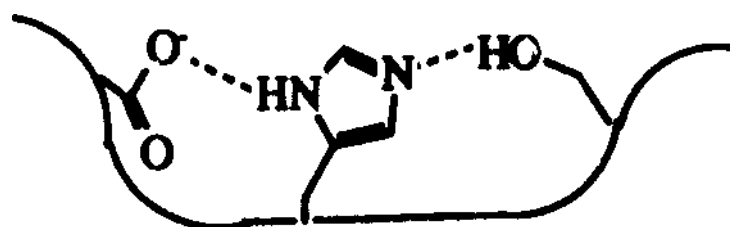


Figure 1

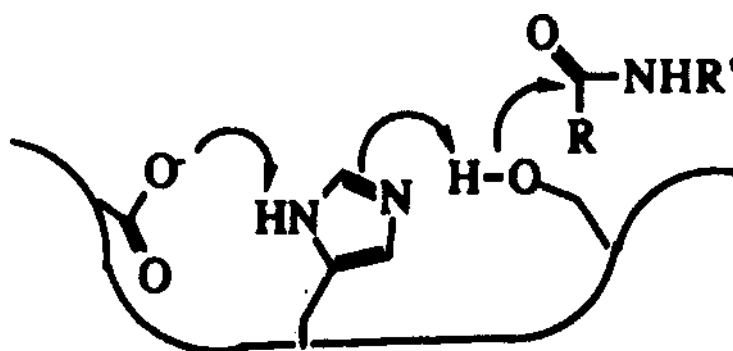


Figure 2

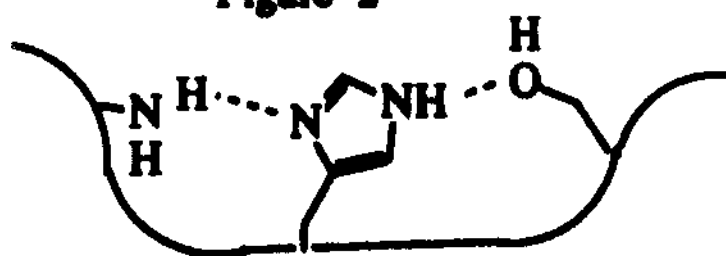


Figure 3

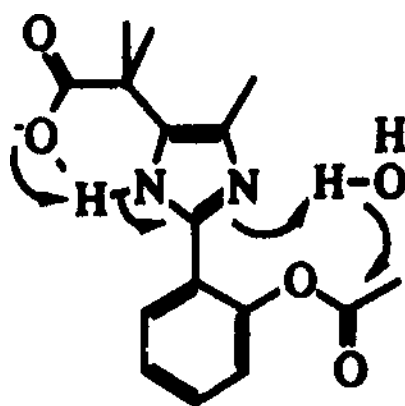


Figure 4

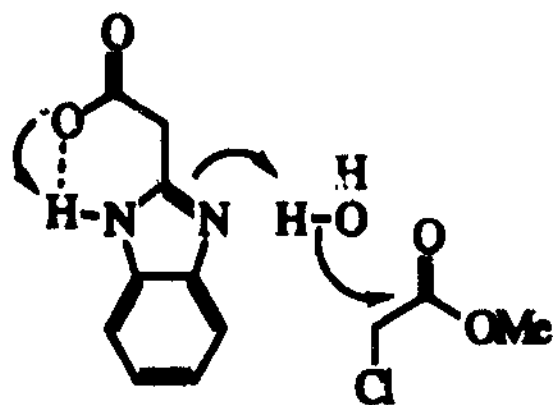


Figure 5



Figure 6

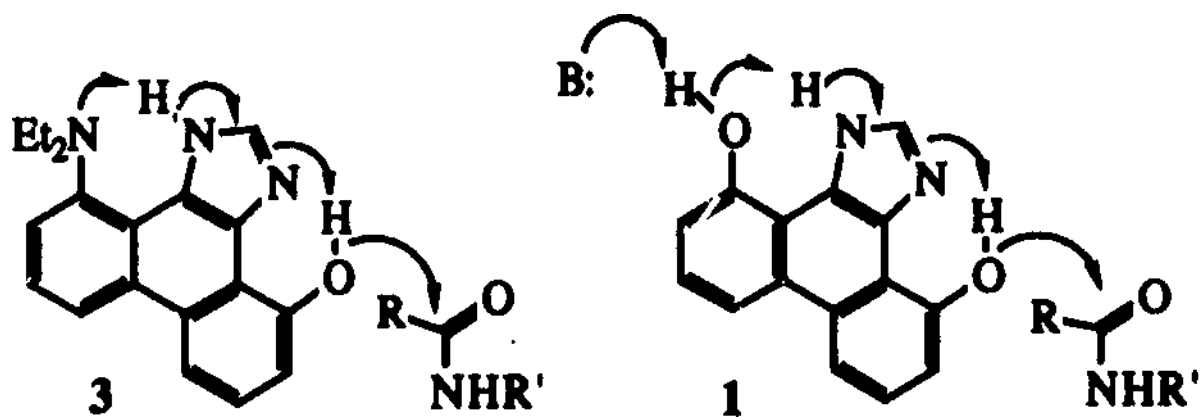
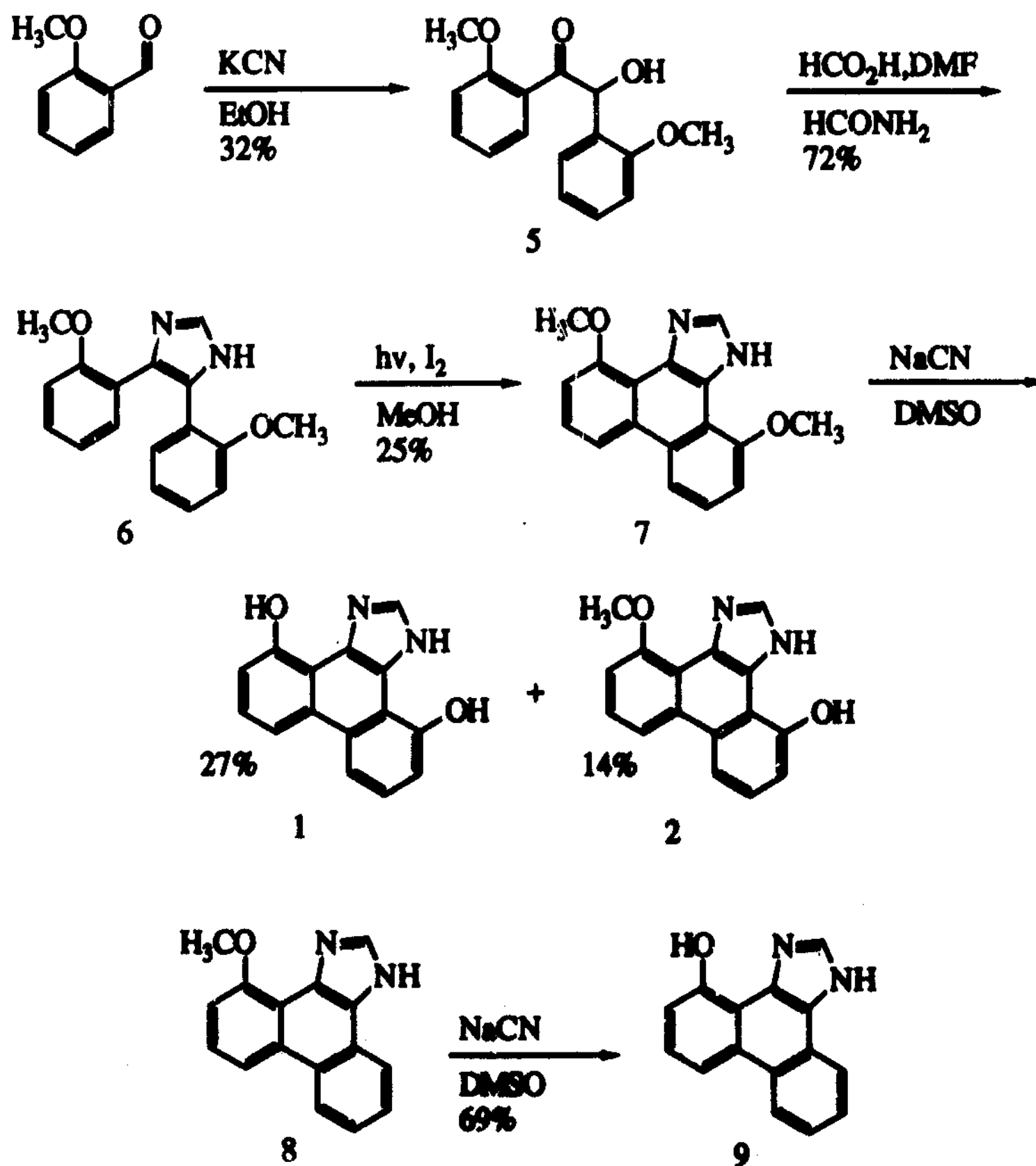
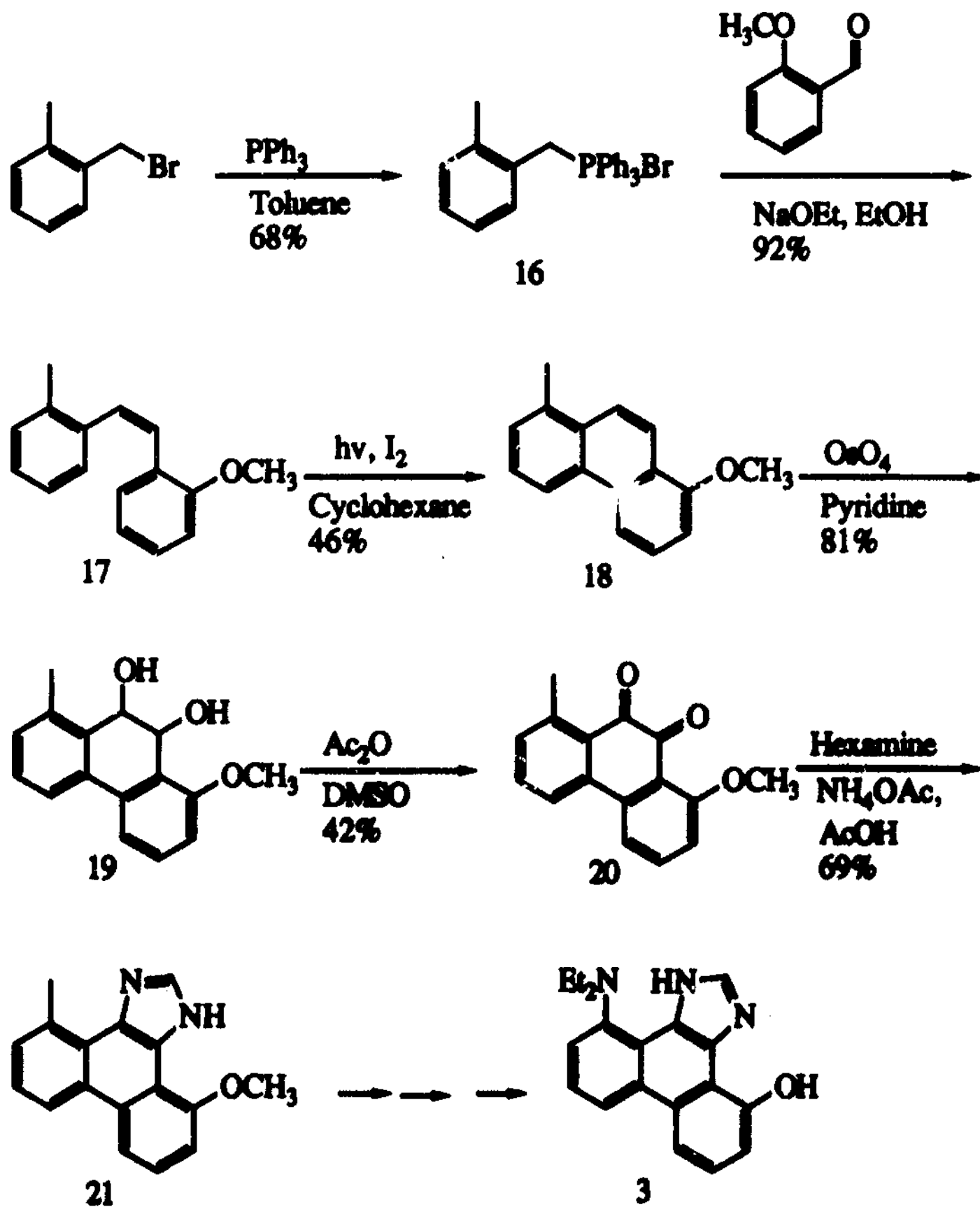


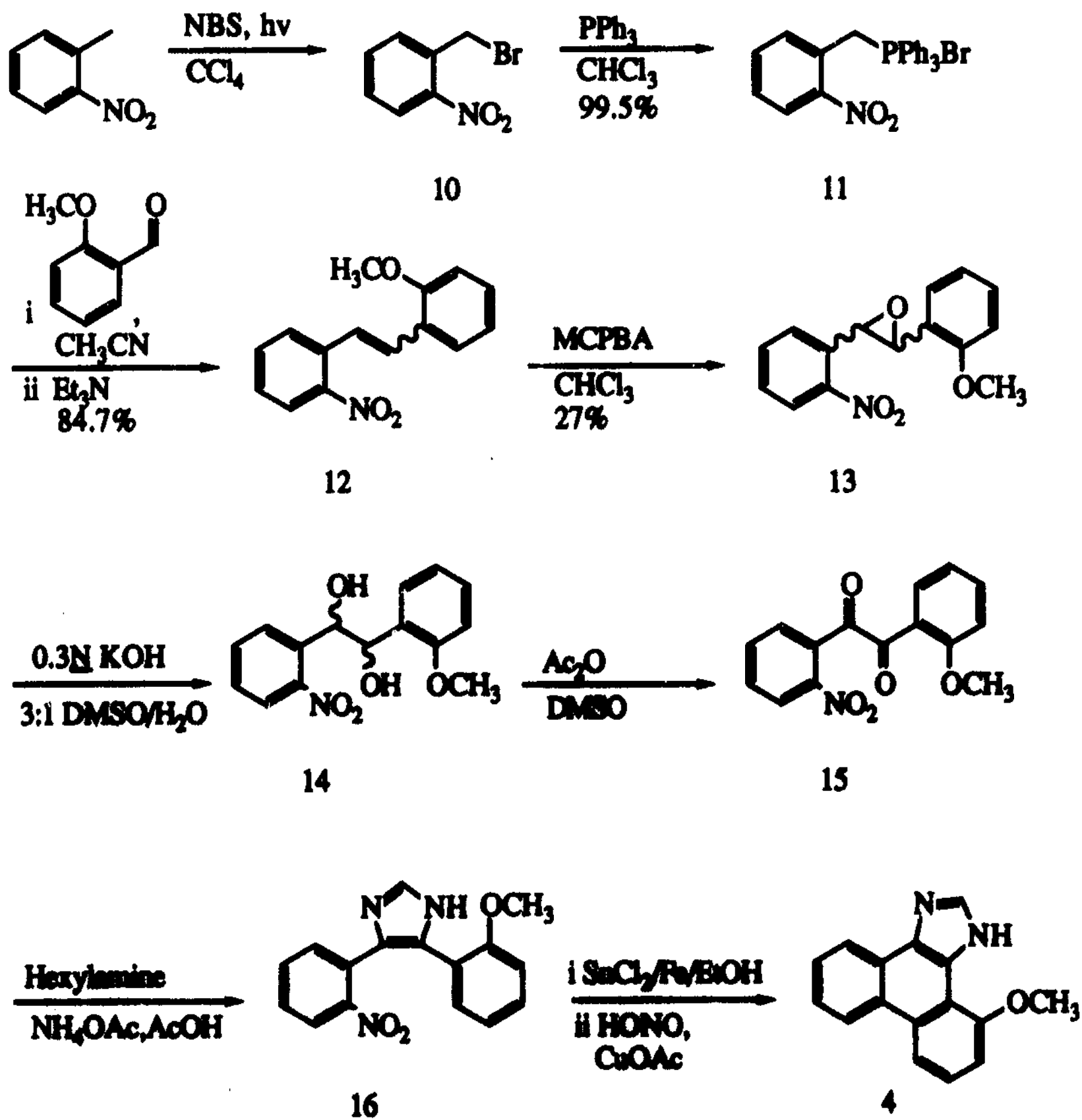
Figure 7



Scheme 1



Scheme 2



Scheme 3

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